DNA OF BACTERIOPHAGE PM2: A CLOSED CIRCULAR DOUBLE-STRANDED MOLECULE

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Abstract.—The DNA molecules isolated from mature bacteriophage PM2 are closed double-stranded rings of a molecular weight of six million. Direct evidence for the circularity and supercoiling of PM2 DNA was obtained by electron microscopy. Other properties, such as sedimentation behavior at neutral and alkaline pH, unusually high buoyant density in alkaline CsCl, and stability upon heating, are consistent with this structure. Only the four common nucleotides are found after hydrolysis and chromatographic analysis of PM2 DNA.

Introduction.—Some properties of the DNA of bacteriophage PM2 have been previously described. The high S value obtained for the DNA after disruption of the virus (which if interpreted as that of a linear DNA would have accounted for more DNA than that contained in the infective particles)[1] and the sedimentation behavior in neutral and alkaline sucrose gradients[2] could be easily explained if bacteriophage PM2 DNA were a closed double-stranded circular molecule.

An approximate molecular weight of 5.1×10^6 was calculated from the sedimentation coefficient observed in sucrose gradients (assuming circularity). Since a circular closed double-stranded DNA of such size present in a mature bacteriophage will be useful in the characterization of these molecules and in studies of DNA replication, we report here a further study of the properties of this DNA. These indicate that PM2 DNA is indeed a circular closed double-stranded DNA. More accurate determination of the sedimentation coefficient and measurements of the contour length of the molecules by electron microscopy suggest that 6.0×10^6 is a more accurate estimate of the molecular weight.

Materials and Methods.—Isolation of PM2 DNA: DNA was extracted from virus grown and purified as previously described;[1] 0.1 to 4.0 mg of purified bacteriophage PM2 in 1 to 2 ml of 1 M NaCl, 0.02 M Tris, pH 8.1, are dialyzed against BE (BE is a solution of 0.1 M NaCl, 0.02 M Tris, 0.001 M EDTA, pH 7.5). After dialysis the suspension is made 0.5% in Sarkosyl (Sarkosyl NL 30, Geigy Industrial Chemicals, New York, N. Y.) and left at room temperature for 5 min until the virus suspension clarifies completely. The solution is then extracted twice with equal volumes of phenol saturated with BE. To recover any DNA remaining in the phenol layer and interphase, the phenol layers are reextracted with a half volume of BE. The aqueous layers are then pooled and dialyzed against BE. The ratio of absorbance at 260 mμ to that at 280 mμ of the viral DNA's thus extracted is between 1.9 and 2.0. Recovery of DNA is about 90%.

Velocity sedimentation: Analytical band centrifugation velocity experiments were performed according to the method of Vinograd *et al.*[3] The sedimentation solvent was 3 M CsCl in sodium phosphate, 0.10 M, pH 7.6. Sedimentation was done at various speeds at 20°C. For sedimentation at alkaline pH, the solvent was 3 M CsCl in 0.05 M sodium phosphate, pH 12.3. The data were adjusted by the least-squares fit in a computer and corrected for the effects of the CsCl solution according to the technique of Bruner and Vinograd.[4]

Equilibrium sedimentation: *Micrococcus lysodeikticus* DNA and crab dAT (from *Cancer anartennarius* sperm), used as density markers, were sedimented to equilibrium with PM2 DNA according to the method of Schildkraut, Marmur, and Doty.[5] Densities of 1.731 gm cm^{-3} for *M. lysodeikticus* DNA[5] and of 1.680 gm cm^{-3} for crab dAT[6] were assumed for calculation of the buoyant density of PM2 DNA. Densities of 1.726 gm cm^{-3} for *M. lysodeikticus* DNA[7] and 1.6696 for crab dAT DNA[8] were assumed for calculation, using the density gradient equation in reference 9. The equation of Vinograd and Hearst[9] was also used for calculation of the alkaline density gradient which was made with CsCl in 0.1 *M* Na_3PO_4. The buoyant density assumed for crab dAT, used as a reference marker, on the alkaline gradient was 1.733 gm cm^{-3} (Vinograd, J., personal communication).

The Beckman model E ultracentrifuge with an automatic photoelectric scanner was used for the velocity and equilibrium sedimentation experiments.

Electron microscopy: DNA was spread on 0.15 *M* ammonium acetate and shadowed essentially according to the procedure of Kleinschmidt and Zahn.[10] The grids were shadowed, while rotating, with platinum-palladium and examined in a Phillips EM 200 electron microscope with a magnification of 8000×. Contour lengths were measured on tracings made on a Nikon shadowgraph at 20×. Total magnification was calculated with a grating replica with 54,865 lines per inch. In some experiments the replicative form of bacteriophage ϕX174 DNA was added to PM2 DNA as an internal standard for the contour length measurements.

Melting temperature: The melting temperature of PM2 DNA in 0.15 *M* NaCl, 0.015 *M* sodium citrate, pH 7.0, was calculated from the absorbance melting profile of nicked PM2 DNA (isolated from a sucrose gradient of a several-months-old preparation). The experiments with closed circular PM2 DNA were performed with a recently purified DNA with less than 3% nicked rings as seen in band sedimentation velocity.

Nucleotide dialysis: 5 to 10 μg of DNA in 0.2 ml of BE was dialyzed against 0.1 *M* Tris, pH 7.6. The dialyzed DNA was made 0.05 *M* in $MgSO_4$ and incubated for 2 hr with 20 μg of deoxyribonuclease I (Worthington Biochemical Corporation, Freehold, New Jersey) at 37°C. After that time 0.1 ml of 0.2 *M* glycine-HCl buffer, pH 9.2, and 10 μg of venom phosphodiesterase (Worthington Biochemical Corporation) were added. Incubation was continued for 2 hr. Chromatographic analysis of the nucleotides was carried out on a Picker Nuclear LCS 1000 nucleic acid analyzer.

Results.—Band centrifugation: The sedimentation velocity pattern for phenol-extracted PM2 DNA consists of one component with a sedimentation coefficient of 26.3*S*. When this DNA is sedimented in an alkaline gradient, 3 *M* CsCl in 0.05 *M* Na_3PO_4, one component is also observed but with an uncorrected *s* value of 48. However, the patterns for aged DNA preparations or for DNA extracted from purified phage several weeks old show the appearance of a second component with an $s_{20,w}$ of 21.2*S* at neutral pH. The appearance of this slow-sedimenting DNA is probably due to some hydrolytic activity.

These sedimentation properties have been previously described (Espejo and Canelo[2]); the differences between the values of the sedimentation coefficients obtained earlier and those reported here are probably due to the inaccuracy of the method previously used.

If it is assumed that the component sedimenting with an $s_{20,w}$ of 21.2*S* is a circular double-stranded DNA with one or more scissions in one strand, a molecular weight of 6.18×10^6 for PM2 DNA can be calculated by using the equation of Gray, Bloomfield, and Hearst.[11] If the equation of Opschoor *et al.*,[12] also relating sedimentation coefficient of nicked circular DNA and molecular weight, is used, a value of 6.05×10^6 is obtained.

Electron microscopy: Direct evidence for the circularity and supercoiling of PM2 DNA was obtained by electron microscopy. Figure 1 shows superhelical and relaxed PM2 DNA. The average contour length observed for 100 relaxed molecules was 3.02 $\mu \pm 0.11$. If a density of 1.96×10^3 daltons/mμ is assumed,[13, 14] a molecular weight of 5.91×10^6 for PM2 DNA is obtained from its contour length. The contour length measured in ϕX174 replicative form DNA, added to some preparations as an internal standard, was $1.67 \mu \pm 0.08$ from which a molecular weight of 3.27×10^6 is obtained. The close agreement between the molecular weight obtained for the replicative form of ϕX174 with those reported (see R. L. Sinsheimer[15]) suggests that the molecular weight calculated for PM2 DNA from its contour length is close to the actual value.

Buoyant density, melting temperature, and nucleotide analysis: The buoyant density of PM2 DNA in CsCl calculated according to Schildkraut *et al.*[5] is 1.702 gm cm^{-3}, which would reflect a guanine-cytosine of 43 per cent if PM2 DNA contains the four common bases. If the more recently reported values for the buoyant density of the reference markers (1.726 gm cm^{-3} for *M. lysodikticus*[7] and 1.6696 gm cm^{-3} for crab dAT (Vinograd, J., personal communication) and

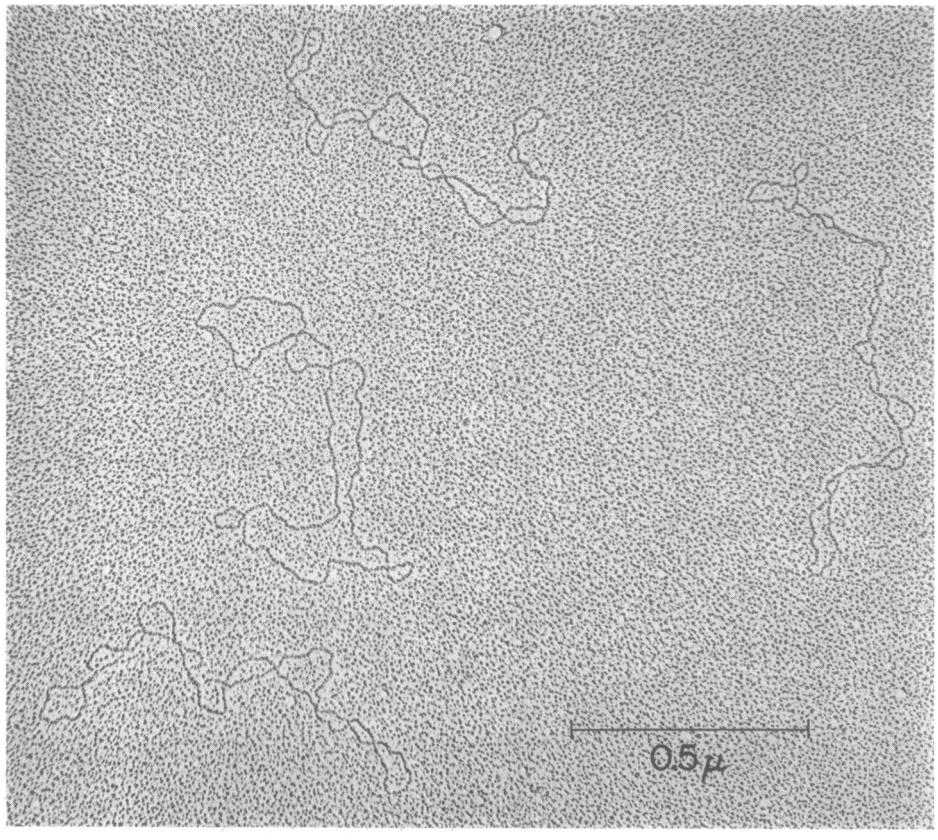

FIG. 1.—DNA of bacteriophage PM2.

the density gradient equation reported by Vinograd and Hearst[9] are assumed, a buoyant density of 1.693 gm cm^{-3} for PM2 DNA is obtained.

The density of PM2 DNA in alkaline CsCl is 1.776 gm cm^{-3}. The pattern observed after sedimentation to equilibrium of nicked PM2 DNA consists of one component with a density of 1.760 gm cm^{-3}, suggesting that the complementary strands have the same buoyant density in alkali (presumably reflecting similar guanine plus thymine content in both strands[3]).

The melting temperature of nicked PM2 DNA in 0.15 *M* sodium chloride, 0.015 *M* sodium citrate, pH 7.0, is 86.4°C, a temperature lower than that previously reported (87.5°C, Espejo and Canelo[1]), when probably a mixture of closed and nicked DNA was used. Intact PM2 DNA does not melt upon heating until 102°C, although a small linear increase is observed at temperatures above 90°C. The melting temperature of the nicked form corresponds to a guanine-cytosine content of 42 per cent according to Marmur and Doty,[16] in close agreement with that calculated from the buoyant density.

Chromatographic analysis of the nucleotides obtained after enzymatic hydrolysis of PM2 DNA shows that it contains adenylic, guanylic, cytidylic, and thymidylic acid, and no indication of unusual nucleotides.

Discussion.—The DNA isolated from mature bacteriophage PM2 is found to consist of circular closed molecules with superimposed tertiary turns, as previously suggested from its sedimentation properties.[2]

The molecular weight of this DNA is 6.0×10^6 based on both the 3.02-μ contour length of the DNA measured in the electron microscope and the sedimentation coefficient of the nicked PM2 DNA. The close agreement of the molecular weight calculated from these parameters (6.18×10^6 or 6.05×10^6 from $s_{20,w}$ and 5.91×10^6 from the contour length) permits confidence in this value.

A molecular weight of 5.2×10^6 would be obtained from the $s_{20,w}$ of PM2 DNA if the experimentally obtained correlation for supercoiled DNA and molecular weight[17] were used. As this value differs considerably from the value of 6×10^6 previously concluded, the discrepancy may reflect a difference in the extent of tertiary twist.

No atypical properties of PM2 DNA were found; the behavior after denaturation, melting profile, buoyant density at neutral and alkaline pH, and base composition are those expected for a circular closed DNA composed of the four common nucleotides.[18]

The natural occurrence of closed circular DNA with superimposed tertiary turns is already an old concept. However, even though it has been shown in animal viruses[19, 20] and in the vegetative forms of several bacterial viruses[21–23] its existence in a mature bacteriophage has not been previously reported. The existence of a supercoiled DNA with a molecular weight of 6×10^6 in bacteriophage PM2 offers a relatively simple system for further studies on DNA replication and the significance of circularity and supercoiling.

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